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Standard Niskin and Van Dorn bottles inhibit phytoplankton photosynthesis in Lake Michigan

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Introduction

The routine collection of water for primary production experiments may result in contamination of the water sample through the introduction of metals or other contaminants (FITZWATER et al. 1982, CHAVEZ & BARBER 1987, WILLIAMS & ROBERTSON 1989). These problems were first discovered in the early 1980s, and focused on the possibility of metal contamination (FITZWATER et al. 1982). Other investigators noted reduced photosynthetic rates when water was collected with standard Niskin bottles (CHAVEZ & BARBER 1987, WILLIAMS & ROBERTSON 1989), and suggested that the problem may not be metal contamination. WILLIAMS & ROBERTSON (1989) suggested that the central rubber cord of the Niskin bottle was the source of contamination. Since these studies, it has been customary for many scientists to use modified Niskin (all rubber parts replaced with silicone or Teflon-coated parts) or Go-Flo bottles for collecting water in primary production studies. While these precautions are common in oceanic research, many limnologists continue to collect water samples for primary production experiments with standard Niskin (e.g. SMITH et al. 1998, MARWOOD et al. 2000) or Van Dorn bottles (e.g. LAMPMAN & MAKAREWICZ 1999, CARIGNAN et al. 2000). A relatively recent text on limnological methods suggests that Van Dorn bottles are well suited for collecting water for primary productivity experiments (WETZEL & LIKENS 1991).

The purpose of this study was to compare standard or conventional techniques of water collection with so-called 'clean' techniques that involve the use of Go-Flo or modified Niskin bottles in a freshwater environment.

Materials and methods

Water was collected at two stations (110 m and 45 m) in Lake Michigan, located off Muskegon, MI. Two research vessels, the R/V *Laurentian* and the R/V *Shenehon*, were used to collect water. Since each vessel has a long history of Great Lakes research, and both have large Niskin bottle racks, Niskin bottles (5-L bottles) on these boats were used as the standard Niskin bottles (SN). The same Niskin bottles were not used for all experiments; rather, bottles were taken randomly off the racks. Additionally, three Niskin bottles were removed from the R/V *Shenehon* and were modified by replacing the buna-N rubber O-rings with silicone O-rings, and the latex rubber connecting cord with a Teflon-coated spring (FITZWATER et al. 1982). These modifications were made to reduce the contact of the collected sample with rubber parts. These bottles will be referred to as modified Niskin bottles (MN). Standard Van-Dorn bottles (VD) were also used to collect water. On one occasion, a 30-L Go-Flo bottle was used to collect water. A Go-Flo bottle (GF) is routinely used to collect contamination-free samples, and is considered the standard for clean water collection.

Because the metal hydroline used to suspend sampling bottles may produce metal contamination, Kevlar and nylon sampling lines were used on two occasions. The Kevlar sampling line was used with the Go-Flo sampling bottles in the first experiment, and nylon line was used in a later experiment. Kevlar and nylon lines were spooled on plastic-wrapped hydrodrums. A Teflon-coated messenger was used with Kevlar and nylon hydrolines. Standard metal messengers were used with metal hydrolines.

All sampling bottles were cleaned with 10% HCl prior to use, and then rinsed several times with DDW. While the sampling bottles were suspended on the hydroline, they were allowed to equilibrate with the lake water for several minutes. Immediately after collection, water was transferred to 2- or 4-L polycarbonate bottles that had been cleaned with liquinox, soaked in 10% HCl overnight, and then rinsed at least five times in DDW. DDW was left in the bottles during storage. These bottles were rinsed several times with sample water. Water from these polycarbonate bottles was dispensed into 250-mL

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polycarbonate incubation bottles (triplicate bottles for each treatment) that had been cleaned and stored in the same way as the larger polycarbonate bottles. These incubation bottles were then injected with a clean ^{14}C stock (prepared as described in FITZWATER et al. (1982)), and incubated for 4–7 h in a Percival incubator at ca. 100 µmol photons m^{-2} s^{-1}. Following this incubation, samples were filtered onto 0.2-µm membrane filters under low vacuum, decontaminated with 50 µL of 0.5 N HCl in scintillation vials (LEAN & BURNISON 1979), and then counted in a liquid scintillation counter.

Results

Experiment 1

The purpose of the first experiment was to compare various types of water samplers with the oceanographic standard for primary production experiments: a Go-Flo bottle secured on Kevlar line and tripped with a Teflon-coated messenger (GFK). This clean sampling technique eliminates the possibility of metal and rubber contamination. The other three treatments included a modified Niskin bottle on a Kevlar line (MNK), a modified Niskin bottle on a metal hydroline (MNM), and a standard Niskin bottle on a metal hydroline (SNM). The only water collection treatment that produced significant differences in photosynthesis was the SNM, which was significantly lower than all other treatments (20% decrease; $P < 0.05$; Fig. 1). Photosynthetic rates for the GFK, MNK, and MNM treatments varied by <5%. Moreover, the similarity between MNK and MNM treatments suggests that the type of hydroline and messenger did not significantly affect the rate of photosynthesis. As a result of the similarity between modified Niskin and Go-Flo bottles, the modified Niskin bottle replaced the Go-Flo as the control sampling bottle.

Experiment 2

In this experiment there were three treatments: a modified Niskin bottle (MNM), a standard Niskin bottle (SNM), and a standard Van Dorn bottle (VDM). All bottles were suspended on a metal hydroline. Highest photosynthetic rates were found with the MNM treatment and significantly lower rates were recorded with the SNM (28% decrease, $P < 0.05$), and the VDM treatments (65% decrease, $P < 0.05$; Fig. 2). This experiment verified the results of the earlier experiment, and extended the possible inhibition results to Van Dorn samplers.

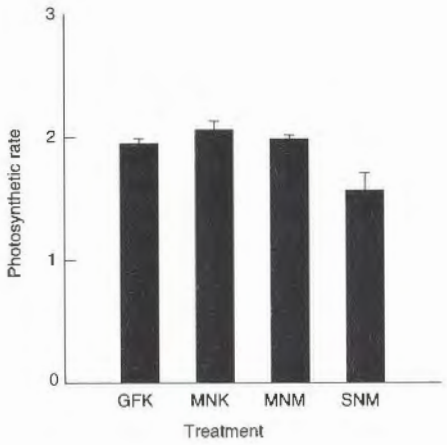

Fig. 1. Photosynthetic rate (mg C m^{-3} h^{-1}) in water collected with three water samplers and two hydrolines. The water samplers were: GFK, Go-Flo bottle on Kevlar hydroline; MNK, modified Niskin bottle on Kevlar hydroline; MNM, modified Niskin bottle on metal hydroline; SNM, standard Niskin bottle on metal hydroline.

Experiment 3

A third experiment was conducted to further verify the inhibition caused by standard Niskin and Van Dorn samplers, and to eliminate the possibility that metal hydrolines were the cause of photosynthetic inhibition. Modified Niskin (MNM for metal hydroline, MNN for nylon hydroline), standard Niskin (SNM for metal hydroline, SNN for nylon hydroline), and Van Dorn (VDM for metal hydroline and VDN for nylon hydroline) bottles were used on metal and nylon hydrolines. The nylon hydroline replaced the Kevlar line because the Kevlar line was not available. No significant difference in photosynthetic rates was found between the two hydrolines ($P > 0.05$; Fig. 3), but significant decreases were noted with standard Niskin and Van Dorn bottles on both lines ($P < 0.05$). In this experiment, standard Niskin and Van Dorn bottles produced similar decreases in pho-

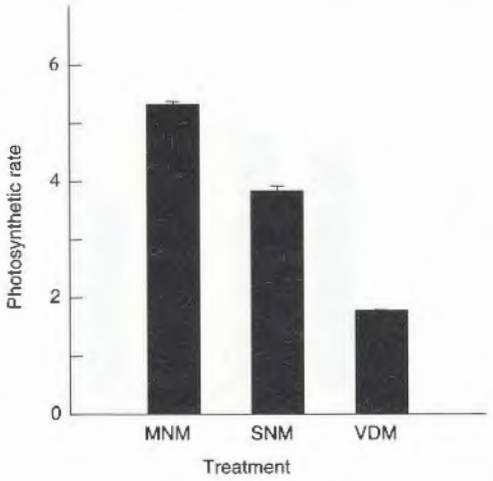

Fig. 2. Photosynthetic rate (mg C m^{-3} h^{-1}) in water collected with three water samplers. The water samplers were: MNM, modified Niskin bottle on metal hydroline; SNM, standard Niskin bottle on metal hydroline; VDM, Van Dorn bottle on metal hydroline.

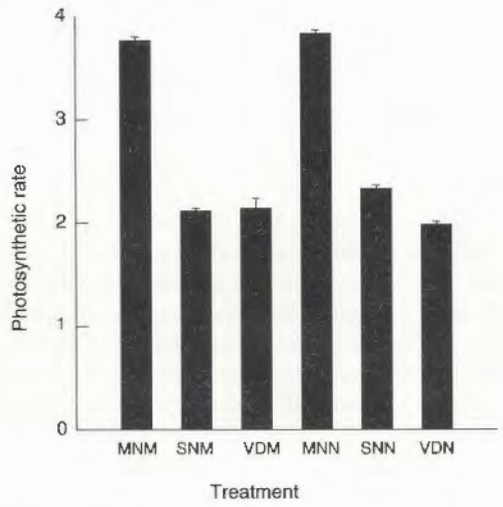

Fig. 3. Photosynthetic rate (mg C m^{-3} h^{-1}) in water collected with three water samplers and two hydrolines. The treatments were: MNM, modified Niskin bottle on metal hydroline; SNM, standard Niskin bottle on metal hydroline; VDM, Van Dorn bottle on metal hydroline; MNN, modified Niskin bottle on nylon hydroline; SNN, standard Niskin bottle on nylon hydroline; VDN, Van Dorn bottle on nylon hydroline.

tosynthetic rates, ranging from 40 to 50% of modified Niskin bottle rates (Fig. 3).

Experiment 4

Because all of the earlier sampling involved standard collections of water aboard large limnological vessels where water casts can take several minutes (5–10 min is typical), it was decided to examine the effects of sampling time, or the time that the water spent in the sampling bottles, on photosynthetic rates. Two types of water samplers were used, a modified Niskin (MNM) and the standard Niskin (SNM). Water samples were left in the individual collection bottles for 1–60 min before they were dispensed into the incubation bottles. Significant decreases were noted for all time periods with the SNM treatment as compared to the MNM treatment ($P < 0.05$; Fig. 4); moreover, the rate of inhibition increased with time. At 1 min confinement, the SNM treatment produced a 16% decrease in photosynthetic rate, whereas with a 60-min confinement, the SNM treatment produced an 80% decrease in the photosynthetic rate (Fig. 4).

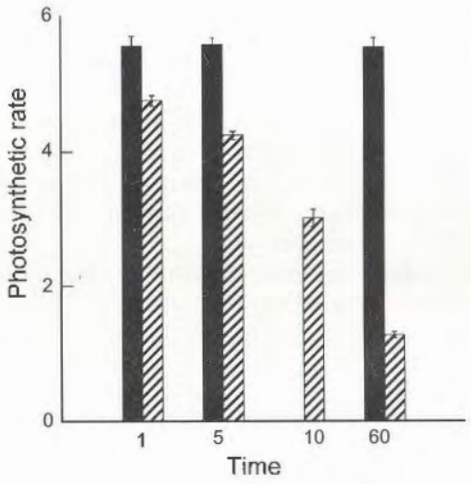

Fig. 4. Photosynthetic rate (mg C m^{-3} h^{-1}) vs. incubation time in water samplers. Two water samplers were used (solid bars, modified Niskin bottle; hatched bars, standard Niskin bottle). Collected water was allowed to incubate in the sampling bottles for 1–60 min before the photosynthetic rate was measured.

Experiment 5

The final experiment was conducted to determine which part(s) of the Niskin and Van Dorn sampling bottles may be inhibiting the photosynthetic rate. Three parts of a Niskin bottle (buna-N rubber O-rings, latex rubber connecting cord and PVC housing) and two parts of a Van Dorn bottle (latex rubber connecting cord, and PVC housing) were suspended for 10 min in water collected with a modified Niskin bottle. The PVC housing of the Niskin and Van Dorn bottles (NH and VDH treatments) did not produce any significant differences in photosynthetic rate as compared to control water (MNM) in either case ($P > 0.05$; Fig. 5). However, both of the connecting cords (NC and VDC treatments) and the O-rings (NO treatment) produced significantly lower photosynthetic rates. The O-ring incubation produced the largest decrease, 53%, whereas the connecting cords produced decreases of 16–20%.

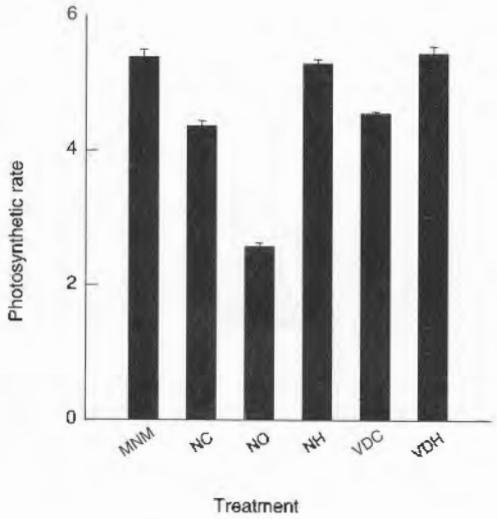

Fig. 5. Photosynthetic rate (mg C m^{-3} h^{-1}) for water collected with a modified Niskin bottle (MNM) and incubated with various parts of standard Niskin and Van Dorn bottles. The treatments were: MNM, control water; NC, latex rubber cord of Niskin bottle; NO, rubber O-ring of Niskin bottle; NH, PVC housing of Niskin bottle; VDC, latex rubber cord of Van Dorn bottle; VDH, PVC housing of Van Dorn bottle.

Discussion

Standard Niskin and Van Dorn bottles should not be used to collect water for phytoplankton photosynthesis experiments in Lake Michigan. In all experiments, water collected with these water samplers exhibited significantly lower photosynthetic rates. The photosynthetic rate decrease was proportional to the amount of time the water spent in the sampling bottles. The present study extends previous work in marine environments (e.g. WILLIAMS & ROBERTSON 1989) to a freshwater environment, and suggests that water for all primary production experiments should be collected with Go-Flo or modified Niskin bottles.

Even though the present experiments were limited, the source of contamination appeared to be the rubber found in the O-rings and connecting cord. The latex rubber cords from both sampling bottles and a buna-N rubber O-ring from a Niskin bottle significantly reduced the photosynthetic rates, whereas the PVC housing from both bottles did not. This reduction of photosynthesis is similar to that reported by WILLIAMS & ROBERTSON (1989), using the latex rubber cords from Niskin bottles with natural communities from the Indian Ocean. WILLIAMS & ROBERTSON (1989) placed the latex rubber cord of a Niskin bottle in water collected with a Go-Flo bottle and noted an 80% reduction in the photosynthetic rate.

Some earlier investigators have attributed the decrease in photosynthetic rate to metal toxicity when using standard Niskin bottles (FITZWATER et al. 1982, MARRA & HEINEMANN 1987). However, in the present study, no evidence of metal toxicity was found. This conclusion was based on the similarity of photosynthetic rates from water collected with modified Niskin bottles on metal hydrolines with metal messengers to rates from water collected with Go-Flo or modified Niskin bottles on synthetic lines (Kevlar or nylon) with Teflon-coated messengers. If metal toxicity was significant, it would have been expected that the treatments with metal hydrolines and messengers would have produced lower photosynthetic rates.

The present results likely have much broader

application than simply phytoplankton photosynthetic rates in Lake Michigan. It is likely that photosynthetic rates will decrease in many freshwater environments if water is collected with standard Niskin or Van Dorn bottles. Additional experiments have been performed in smaller Michigan lakes, and in most cases, similar reductions in the photosynthetic rate were observed. Moreover, the negative effects of using standard Niskin or Van Dorn bottles likely extend far beyond photosynthetic rates. WILLIAMS & ROBERTSON (1989) noted a decrease in chlorophyll concentrations in water collected with standard Niskin bottles. PRICE et al. (1986) noted that latex rubber suspended in clean water caused cellular death for many phytoplankton species. This effect was species specific, as not all species were killed. Moreover, phytoplankton are not the only group of planktonic organisms affected by latex rubber. Thymidine incorporation by bacteria was significantly reduced in the presence of latex rubber, and the survival of the crustacean zooplankton, *Acartia clausii*, was substantially lower when incubated in the presence of latex rubber (exposure lasted for several days; PRICE et al. 1986). Thus, it is probably a wise practice to use modified Niskin or Van Dorn bottles for all biological sampling.

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References

CARIGNAN, R., PLANAS, D. & VIS, C., 2000: Planktonic production and respiration in oligotrophic shield lakes. – *Limnol. Oceanogr.* 45: 189–199.

CHAVEZ, F. P. & BARBER, R. T., 1987: An estimate of new production in the equatorial Pacific. – *Deep-Sea Res.* 34: 1229–1243.

FITZWATER, S. E., KNAUER, G. A. & MARTIN, J. H., 1982: Metal contamination and its effect on primary production measurements. – *Limnol. Oceanogr.* 27: 544–551.

LAMPMAN, G. G. & MAKAREWICZ, J. C., 1999: The phytoplankton zooplankton link in the Lake Ontario food web. – *J. Great Lakes Res.* 25: 239–249.

LEAN, D. R. S. & BURNISON, B. K., 1979: An evaluation of errors in the ^{14}C method of primary production measurement. – *Limnol. Oceanogr.* 24: 917–928.

MARRA, J. & HEINEMANN, K. R., 1987. Primary production in the North Pacific Central Gyre: some new measurements based on C-14. – *Deep-Sea Res.* 34: 1821–1829.

MARWOOD, C. A., SMITH, R. E. H., FURGAL, J. A., CHARLTON, M. N., SOLOMON, K. R. & GREENBERG, B. M., 2000: Photoinhibition of natural phytoplankton assemblages in Lake Erie exposed to solar ultraviolet radiation. – *Can. J. Fish. Aquat. Sci.* 57: 371–379.

PRICE, N. M., HARRISON, P. J., LANDRY, M. R., AZAM, F. & HALL, K. J. F., 1986: Toxic effects of latex and Tygon tubing on marine phytoplankton, zooplankton and bacteria. – *Mar. Ecol. Prog. Ser.* 34: 41–49.

SMITH, R. E. H., FURGAL, J. A. & LEAN, D. R. S., 1998: The short-term effects of solar ultraviolet radiation on phytoplankton photosynthesis and photosynthate allocation under contrasting mixing regimes in Lake Ontario. – *J. Great Lakes Res.* 24: 427–441.

WETZEL, R. G. & LIKENS, G. E., 1991: *Limnological Analysis*. 2nd Ed. – Springer-Verlag, New York, 391 pp.

WILLIAMS, P. J. LE B. & ROBERTSON, J. I., 1989: A serious inhibition problem from a Niskin sampler during plankton productivity studies. – *Limnol. Oceanogr.* 34: 1300–1305.

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